REMARKS

This Preliminary Amendment, filed concurrently with a Request for Continued Examination, also serves as a full and timely response to the Final Office Action dated October 19, 2006 and the Advisory Action dated April 3, 2007. The filing of this RCE and Amendment is permissible under 37 C.F.R. § 1.114. *See* M.P.E.P. § 706.07(h). A Petition for Extension of Time within the Third Month is filed concurrently herewith.

The present Amendment amends claims 20 and 28 and cancels claims 25-27 and 29 without prejudice or disclaimer in order to further clarify a portion of the scope sought to be patented, and otherwise disputes certain findings of fact made in connection with the rejection of the claims. Support for these amendments can be found variously throughout the specification, including, for example, original claim 20 and previously presented claims 27 and 28. No new matter has been added.

Specification & Abstract

In response to the objections to the Specification under 35 U.S.C. § 132(a), Applicant respectfully asserts that the Amendments to the Specification are appropriate and correct. The Amendments to the Specification do not add new matter, but were made merely to correct errors in the English translation of the text based on the original Japanese text (JP 2002-167920).

Claim Objections

Applicant thanks the examiner for a thorough reading of the claims. In accordance with the examiner's suggestion, claim 28 has been amended to correct minor informalities. Withdrawal of this objection is therefore courteously solicited.

Claim Rejections- 35 U.S.C. § 112

In the Action, claims 20 and 25-29 were rejected under 35 U.S.C. § 112, second paragraph, for alleged indefiniteness. Applicant respectfully traverses this rejection. However, in order to

expedite prosecution, claim 20 has been amended to recite "isolating IgG fraction" and "purifying the isolated IgG fraction."

With the Amendment, the difference between the preparing step and the purifying step is apparent. Withdrawal of this rejection is therefore courteously solicited.

Claim Rejections- 35 U.S.C. § 103

In the Action, claims 20 and 25-29 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kawahara et al., *Quantitative analysis of protein synthesis altered by estrogen in cultured Xenopus liver parenchymal cell*, Develop., Growth and Differ. 23, 599-611 (1981) ("Kawahara") in view of Dunbar et al., *Preparation of Polyclonal Antibodies*, Methods in Enzymology 182, 663-670, (1990) ("Dunbar") and Harlow & Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 283, 285-293, and 331 (1988) ("Harlow"). Claims 20 and 25-29 were further rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Shapiro et al., *In Vitro Translation and Estradiol-17β Induction of Xenopus laevis Vitellogenin Messenger RNA*, The Journal of Biological Chemistry, Vol. 251, No. 10, pp. 310-3111, (1976) ("Shapiro") in view of Kawahara and Harlow. This rejection is respectfully traversed.

The process of the present invention is characterized in that purifying the isolated IgG fraction by affinity purification takes place AFTER adsorption purification using a column. By this method, a polyclonal antibody specific to a frog vitellogenin with high degree of specificity can be obtained and frog vitellogenin can be detected and the quantity thereof can be determined by using the obtained antibody. Applicant would like to point out that no products equivalent to that of the present invention can be produced by combining the methods recited in the cited references. Thus, it is NOT obvious to determine the combination of processes or to designate the order of process indicated in the present invention and the invention should be accordingly be held allowable.

In the present invention, isolating IgG fraction is firstly performed. Because in the case of purification using a column, mixing in of unnecessary proteins caused by anti specific combination occurs, it is necessary to eliminate as much unwanted anti blood serum proteins as possible. Mixing

in of unnecessary proteins can be minimized by firstly isolating IgG fraction as in the present invention.

Furthermore, as shown in the attached experiment, the antibody obtained by performing an affinity purification after an adsorption purification using a column has high antibody value to VTG and low degree of anti specific reaction to normal male blood serum proteins. If the affinity purification is performed without adsorption purification, not only anti-vitellogenin antibody but also various proteins including IgG in blood serum remain in the obtained antibody in an amount that cannot be ignored. These various proteins, including IgG in blood serum, cannot be eliminated by the adsorption purification.

Kawahara describes obtaining vitellogenin specific antisera from blood collected from an immunized rabbit and states that "the prepared anitisera were absorbed with normal male sera in order to obtain vitellogenin specific antisera." Nevertheless, there is no mention of isolating IgG fraction from the antiserum, an adsorption purification using a column, or affinity purification. Adsorption purification described in Kawahara involves adding of male frog blood serum of quantity more than equivalent point to anti-blood serum and eliminating the reaction sediment. Antibody neutralized with frog blood serum proteins will remain in vitellogenin specific anti blood serum obtained by adsorption purification which does not use a column, as disclosed in Kawahara. On the contrary, in the present invention frog blood serum proteins and neutralized antibody are not mixed in antibody obtained by adsorption purification using a column.

Harlow describes an ammonium sulfate fractionation, DEAE, and an affinity purification on an antigen column as methods of purifying antibodies. Furthermore, Harlow describes combining a plurality of purifications. The ammonium sulfate fractionation and DEAE described therein are used when isolating IgG from antiserum as the present invention describes. The affinity purification on an antigen column described in Harlow corresponds to the affinity purification using a column of the present invention. However, Harlow does not describe the adsorption purification using a column.

Dunbar describes preparation of polyclonal antibodies. Dunbar describes obtaining IgG from serum taken from blood collected from a rabbit and purification thereof. Three types of purification,

namely, ammonium sulfate fractionation, DEAE anion exchange chromatograpy, and affinity purification using protein A are described as methods of purifying IgG. However, there is no mention of "an adsorption purification using a column coupled with blood serum of a male frog containing proteins" and "an affinity purification using a column coupled with a frog vitellogenin" as in the present invention.

Shapiro describes producing vitellogenin antigen to immunize a rabbit by using male frogs induced with estradiol-17β and IgG fraction. However, there is no mention of purifying the isolated IgG fraction by adsorption purification using a column and affinity purification. It is not surprising that using vitellogenin antigen produced with male frogs induced with estradiol-17β to a column used in affinity purification that is not mentioned by Shapiro.

The Examiner asserts that it would have been obvious that the present invention might be formed by combining Kawahara, Dunbar and Harlow. However, Applicant considers that the present invention is patentable, even if one were to consider combining the cited references.

As mentioned above, frog blood serum proteins and neutralized antibody remain in vitellogenin specific anti blood serum obtained by adsorption purification not using a column as described on Kawahara. Therefore, no products equivalent to that of the present invention can be produced by the combination of the adsorption not using a column, as described on Kawahara, and the IgG fraction and the affinity purification described on Dunbar and Harlow.

Accordingly, Applicant considers that it is NOT obvious to produce products equivalent to that of the present invention as claimed, after amendment, from a combination of steps recited in the cited references. Furthermore, it is NOT obvious to determine the combination of process steps or to designate the order of process steps indicated in the present invention as claimed, after the amendment

The Examiner asserts on page 8, lines 3-4 of the Office Action that "in this case the use of a "column" to adsorb the polyclonal antisera with normal male sera does not imply any structural difference in the resulting polyclonal antibodies." However, Applicant asserts that the product

obtained by the adsorption purification using a column of the present invention differs from the product obtained by the adsorption purification not using a column, as described in Kawahara for the reasons mentioned above.

In addition, the Examiner asserts that there seems to be no structural difference in the obtained polyclonal antibody specific to a frog vitellogenin, regardless of the order of steps of the adsorption purification and the affinity purification (page 9, line 20 through page 10, line 2 of the Office Action). However, as shown in the attached experimental data (*see Appendix*), the antibody obtained by designating the order of steps in the purification process IS different in its quality.

Furthermore, Applicant would like to explain the designation of vitellogenin induced in male or female frog, as mentioned by Examiner on page 9, in line 20 through page 10, line 2 in the Office Action. If vitellogenin from blood serum of a female frog is used for immune antigen, there is a possibility that antibody directed against combined protein or modified residue vitellogenin-binding protein unique to female etc. may be formed in the antiserum. Such antibodies cannot be eliminated by male serum protein. Especially in the case of vitellogenin from blood serum of a female, a partial disintegration of protein can be seen since vitellogenin always exists in blood serum. This may cause antigency that cannot be seen in normal vitellogenin. Therefore, in a case of an antiserum obtained using purified vitellogenin induced by male serum to an immunizing antigen, a very small amount of protein other than vitellogenin that could be a cause of non-specific antibody becomes protein derived from a male, the very small amount of protein other than vitellogenin that would be a cause of non-specific antibody can be absorbed and eliminated in a step of adsorption purification column coupled with blood serum proteins of a male frog.

The Examiner further asserts that the present invention is not patentable over Shapiro, Kawanara, and Harlow. However, Applicant asserts that the present invention IS patentable even when all the cited references are considered.

As mentioned above, frog blood serum proteins and antibody neutralized by blood serum of a frog remains in vitellogenin specific anti blood serum obtained by adsorption purification not using a column, as described in Kawahara. Therefore, no products equivalent to that of the present

invention can be produced by combination of the adsorption not using column described in Kawahara, IgG fraction as described in Shapiro, and the affinity purification as described in Harlow.

Applicant considers that it is NOT obvious to produce products equivalent to that of the present invention as claimed, after amendment, from a combination of steps recited in the cited references. Furthermore, it is NOT obvious to determine the combination of process steps or to designate the order of process steps indicated in the present invention as claimed, after the amendment.

As described above, in Claim 28, by using purified vitellogenin induced by male serum as an immunizing antigen, a very small amount of protein other than vitellogenin that would be a cause of non-specific antibody becomes protein derived from a male, the very small amount of protein other than vitellogenin that would be a cause of non-specific antibody can be absorbed and eliminated in a step of adsorption purification column coupled with blood serum proteins of a male frog. In this case, by specifying the immunizing antigen and specifying the column in the adsorption purification to be coupled with blood serum proteins of a male frog, a very small amount of proteins other than vitellogenin can be absorbed and eliminated. If the adsorption step and the affinity purification using no column, as described in Kawahara, were performed using vitellogenin induced by a male blood serum as the immunizing antigen by combining Kawanhara, Shapiro and Harlow, the antibody equivalent to that of the present invention could not be obtained.

Shapiro describes producing vitellogenin antigen to immunize a rabbit by using male frogs induced with estradiol-17β and IgG fraction. It is similar to the present invention as described in Claim 28 only at the point where vitellogenin induced from a male frog is used as the immune antigen. As described above, according to Claim 28, by using purified vitellogenin induced by male serum as an immunizing antigen, a very small amount of protein other than vitellogenin that would be a cause of non-specific antibody becomes protein derived from a male, the very small amount of protein other than vitellogenin that would be a cause of non-specific antibody can be absorbed and eliminated in a step of adsorption purification using a column coupled with blood serum proteins of a male frog. In this case, by specifying the immunizing antigen and specifying that the column in the adsorption purification is coupled with blood serum proteins of a male frog, a very small amount

of proteins other than vitellogenin can be absorbed and eliminated. If the adsorption step and the affinity purification using no column, as described in Kawahara, were performed using vitellogenin induced by a male blood serum as the immunizing antigen by combining Kawanhara, Shapiro and Harlow, the antibody equivalent to that of the present invention could not be obtained.

Accordingly, because Kawahara, Dunbar, Harlow, and Shapiro, either alone or in combination, fail to disclose, teach or suggest each and every limitation of claims 20 and 28, a *prima facie* case of obviousness has not been established, and withdrawal of this rejection is respectfully requested. *See, e.g., In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974); *accord.* MPEP 2143.03.

CONCLUSION

In view of the above amendment, Applicant believes the pending application is in condition for allowance.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 18-0013, under Order No. OMY-0041 from which the undersigned is authorized to draw.

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Respectfully submitted,

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Appendix: Supplementary Experimental Data

APPENDIX

Supplementary Experimental Data

Aim of research

In order to demonstrate superiority of anti-VTG antibody prepared by the present application procedure (the pre-adsorption method), reactivity and quality of this antibody were compared with those of antibody prepared by the post-adsorption method (the adsorption purification was performed after the VTG-affinity purification).

Methods

Preparation of antibody

Antibodies were prepared from a single batch of anti-VTG rabbit IgG fraction as follows:

1. Antibody 1 was obtained by the VTG-affinity purification of the adsorbed IgG (the pre-adsorbed antibody).
2. Antibody 2 was obtained by the adsorption purification of the VTG-affinity-purified IgG (the post-adsorbed antibody).
3. Antibody 3 was obtained by the VTG-affinity purification of the IgG.
4. Antibody 4 was obtained by the adsorption purification of the IgG.
5. The IgG (untreated)

Detection of VTG and male Xenopus serum proteins by ELISA

Serially-diluted preparations of antibody 1, 2, 3, and 4 and the IgG were reacted with the following ELISA plates (assay A and assay B) according to the standard procedure described in the present application. The chromogenic detection using HRP-labeled anti-rabbit IgG goat antibody as a secondary antibody was performed by measurement of absorbance at 450 nm.

Assay A: ELISA was performed using plates that were coated with standard VTG (5 ug/ml VTG, 50 ul/well).

Assay B: ELISA was performed using plates that were coated with normal male Xenopus serum (1/100-diluted normal male serum, 50 ul/well).

Results and Discussion

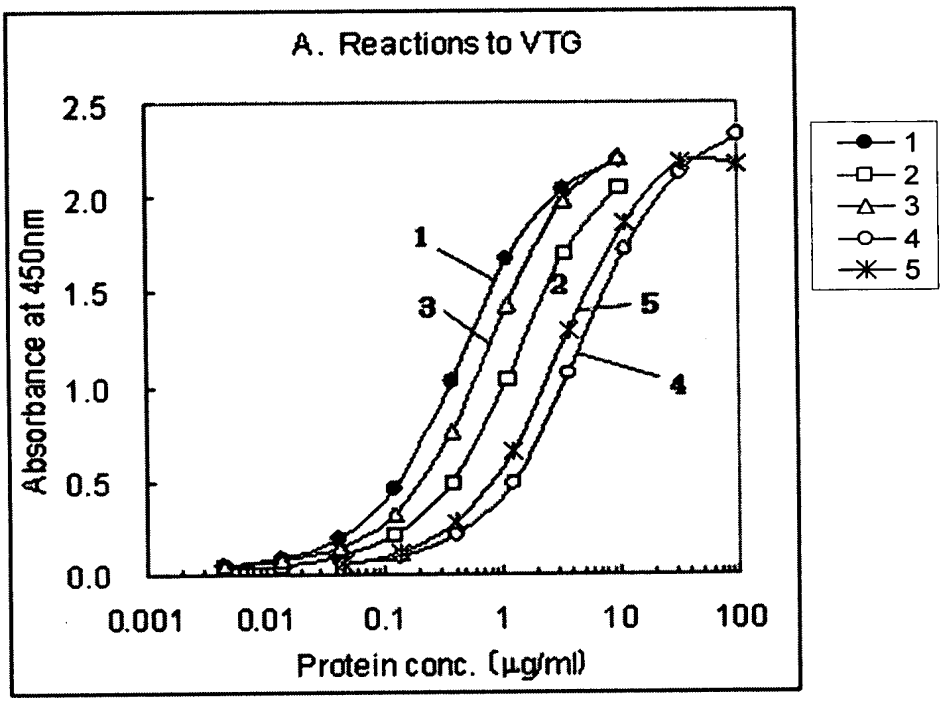

Figure A. Reactions to VTG (Assay A). Numerals marking the reaction curves indicate the antibody or IgG preparations described in Methods Each point represents the mean of 4 determinations.

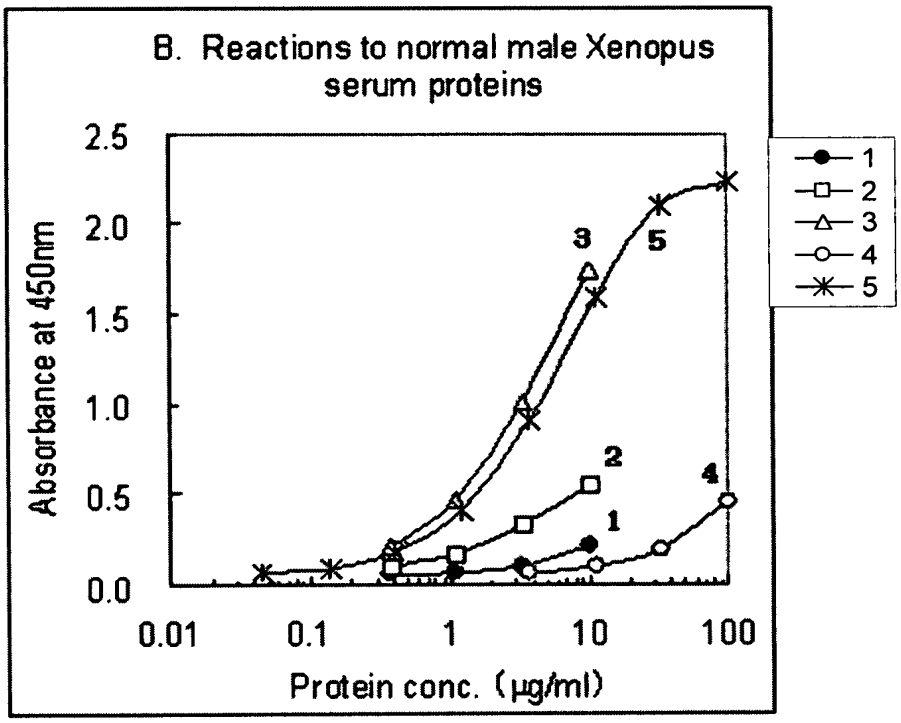

Figure B. Reactions to normal male Xenopus serum proteins (Assay B). Numerals marking the reaction curves indicate the antibody or IgG preparations described in Methods. Each point represents the mean of 4 determinations.

The pre-adsorbed antibody (antibody 1) reacted to VTG at the lowest antibody concentration (Fig. A curve 1) among the preparations tested, but its reactivity to the male serum proteins (MSP) was very low (Fig. B curve 1). On the other hand, the post-adsorbed antibody (antibody. 2) reacted significantly to MSP (Fig. B curve 2). Reactivity of the pre-adsorbed antibody (antibody 1) relative to that of the post-adsorbed antibody (antibody 2) was 3-fold higher in reaction to VTG, but 10-fold lower in reaction to MSP. Therefore, it is considered that specificity of antibody 1 to VTG was 30-fold higher than that of antibody 2. The adsorption purification alone was able to markedly reduce reactivity of the original IgG to MSP (compare curve 4 and curve 5 in Fig. A), but did not increase that to VTG (compare curve 4 with curve 5 in Fig. B). By contrast, although the VTG-affinity purification alone was able to greatly increase reactivity of the original IgG to VTG (compare curve 3 with curve 5 in Fig. A),

reactivity of antibody 3 to MSP was still high (compare curves 3 with curve 5 in Fig. B). This high reactivity to MSP was considered to be largely due to non-specific binding of IgG to the VTG-conjugated affinity column, because the highly phosphorylated protein, VTG, may provide binding sites of IgG. In addition, the column will also bind antibody that are cross-reactive to VTG and MSP, by which the antibody prepared may react to MSP at higher extent than the original IgG does.

Conclusion

An antibody prepared by the pre-adsorption method can react more sensitively and specifically to VTG, compared to the antibody prepared by the post-adsorption method. Thus, the method described in the present application is essential for realizing the highly specific ELISA for VTG.